

Remarks

Amendments

Claim 2 has been amended to recite that the claimed method comprises a step of digesting reagents for polymerase chain reaction (PCR) with restriction endonuclease AluI. Claims 4, 21, and 22 have similarly been amended to recite that the restriction endonuclease is AluI. The specification supports these amendments at page 8, lines 1-3: "One can amplify without contamination or with much reduced contamination by digesting all of the reagents for amplification with a restriction endonuclease. AluI is a preferred enzyme although others can be used." Claim 3, which had recited that "the restriction endonuclease is AluI" also supports these amendments.

Claim 8 has been amended to recite dependency from "claim 2" in place of now canceled "claim 3." This amendment merely addresses a formal matter.

None of these amendments introduce new matter.

The amendments will not require any further search or consideration. The Patent Office has already searched and considered claim 2 for a method of performing PCR which employs AluI restriction endonuclease. Originally filed claim 3, which had depended upon claim 2, recited that "the restriction endonuclease is AluI."

The amendments were not earlier made because they respond to rejections first raised by the Patent Office in the currently pending Office Action. Applicants also believe that the amendments place the claims in condition for allowance.

Entry of these amendments is respectfully requested.

The Rejection of Claims 2 and 4 Under 35 U.S.C. § 102(b)

Claims 2 and 4 have been rejected under 35 U.S.C. § 102(b) as anticipated by Steinman (U.S. Patent No. 5,516,292).

Amended claim 2 recites a method of performing PCR. Reagents for PCR are digested with AluI restriction endonuclease to form digested reagents. The reagents comprise Taq DNA polymerase, deoxynucleotide triphosphates, reaction buffer, and a pair of primers. The AluI restriction endonuclease does not cleave the pair of primers and both primers of the pair of primers have no recognition sites for the AluI restriction endonuclease. The AluI restriction endonuclease, but not the Taq DNA polymerase, is inactivated to form AluI endonuclease-inactivated digested reagents. A test sample is mixed with the AluI endonuclease-inactivated digested reagents to form a mixture. The mixture is subjected to conditions such that any templates present in the test sample which hybridize to both primers of the pair of primers are amplified. The amplification product is detected. A detected amplification product indicates the presence of template which hybridizes to both primers in the test sample. Claim 4 recites that the step of inactivating comprises heating to a temperature which inactivates the AluI restriction endonuclease but not the Taq DNA polymerase.

A reference cited under 35 U.S.C. § 102 must expressly or inherently describe each element set forth in the rejected claim. *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987). Steinman does not anticipate the rejected claims because Steinman does not expressly or inherently teach each element set forth in the rejected claims, *i.e.*, digesting reagents for PCR with AluI restriction endonuclease. The Office Action acknowledges that Steinman does not teach this element of claims 2 and 4. The Office Action states, "Steinman did not specifically teach use of AluI." Office Action at page 5, line 9. Thus, Steinman does not

anticipate claims 2 and 4.

Applicants respectfully request withdrawal of this rejection.

The Rejection of Claims 3, 8-10, 15, and 18 Under 35 U.S.C. § 103(a)

Claims 3, 8-10, 15, and 18 have been rejected under 35 U.S.C. § 103(a) as unpatentable over Steinman in view of DeFilippes (*Biotechniques* 10 (1991):26-30). Claim 3 has been canceled. Thus, the rejection of this claim is moot. Applicants respectfully traverse the rejection as it is applied to claims 8-10, 15, and 18.

Claims 8-10, 15, and 18 all depend from claim 2. Amended claim 2, discussed above, is directed to a method of performing PCR that includes a step of digesting reagents for PCR with AluI restriction endonuclease. Claim 8 recites that the step inactivating is performed at about 65°C for about 20 minutes. Claim 9 recites that the step of detection employs an agarose gel. Claim 10 recites that the amplification product is labeled with ethidium bromide and visualized under ultraviolet light. Claim 15 recites that the primers hybridize to 16S RNA genes. Claim 18 recites that the method of claim 2 further comprises a step of identifying a bacterial species as a source of the templates by amplification of the amplification product using primers which hybridize to a single eubacterial species 16S RNA. DeFilippes is cited as teaching the added recitations in each of these dependent claims.

To reject claims as *prima facie* obvious the Patent Office must meet three criteria:

First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

MPEP § 2143. The *prima facie* case of obviousness of claims 8-10, 15, and 18 must fail because one of ordinary skill in the art would not have combined Steinman and DeFilippes to arrive at the claimed invention, *i.e.*, the *prima facie* case fails to meet the first criterion.

When determining the patentability of a claimed invention which combines two known elements, there must be something in the prior art as a whole to suggest the desirability, and thus the obviousness, of making the combination. *In re Beattie*, 974 F.2d 1309 (Fed. Cir. 1992) (quoting *Lindemann*, 730 F.2d at 1462 (Fed. Cir. 1984)). As a prior art reference must be considered in its entirety, even portions that would lead away from the claimed invention must be considered. When there is such a clear teaching away in the prior art, the prior art would not suggest the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540 (Fed. Cir. 1983).

Independent claim 2, from which claims 8-10, 15, and 18 depend, recites a method of performing polymerase chain reaction that includes a step of "digesting reagents for polymerase chain reaction with AluI restriction endonuclease." As discussed above, Steinman does not teach digesting reagents for PCR with AluI restriction endonuclease. Steinman only generally teaches employing a restriction endonuclease to digest PCR reagents. Steinman teaches, "In another alternative embodiment, at least one restriction endonuclease is added to an amplification reaction mixture solution containing contaminant double stranded DNA." Column 3, lines 31-33.

The Office Action cites DeFilippes as teaching a method of performing PCR which includes a step of digesting the PCR reagents with AluI restriction endonuclease. Office Action at page 5, lines 21-22. DeFilippes teaches that AluI can be employed to digest PCR reagents in a PCR method. However, when DeFilippes is considered as a whole, DeFilippes teaches away

from the use of AluI in such a PCR method because DeFilippes teaches that AluI does not effectively digest PCR reagent mixtures, *i.e.*, AluI does not effectively digest contaminating DNA that may be present in reagents for PCR.

DeFilippes teaches that before employing a restriction endonuclease in his PCR method, the restriction endonuclease should be tested to determine its effectiveness at digesting any contaminating DNA that may be present in the reagents for PCR. DeFilippes teaches, "I incubated a series of PCR mixtures with different restriction enzymes to find those which would inactivate contaminating DNA." Page 26, column 3, lines 22-25 of the Introduction. DeFilippes teaches a test for determining the effectiveness of a restriction endonuclease in his PCR method which includes the following steps:

- restriction endonuclease digesting reagents for PCR including template DNA (to simulate contaminant DNA), primers, deoxyribonucleotides, and restriction endonuclease buffer (page 28, column 1, lines 13-19 of the Materials and Methods (M&M));
- inactivating the restriction endonuclease (page 28, column 1, lines 20-21 of the M&M);
- adding PCR buffer and Taq polymerase to the digested reagents (page 28, column 1, lines 22-24 of the M&M);
- adding template DNA to a portion of the mixture including the PCR buffer and Taq polymerase (page 28, lines 28-29 of the M&M); and
- performing PCR (page 28, lines 31-34 of the M&M).

Amplification of the PCR mixture containing the restriction endonuclease-digested reagents (template DNA simulating contaminant DNA, primers, deoxyribonucleotides, and restriction endonuclease buffer) PCR buffer, and Taq polymerase should not yield any products if the restriction endonuclease effectively cleaves all the contaminant DNA (simulated by template DNA).

DeFilippes teaches that in both a first and a second set of test assays, however, AluI did

not successfully decontaminate the reagents for PCR. DeFilippes teaches of the first set of test assays, "In fact, the digestion with *AluI*, illustrated in Figure 1, was not routinely successful, and in some cases a light band was present at the proper position in lane 7 although no template was added after the inactivation of the *AluI*." Page 28, column 2, line 16 to column 3, line 1.

DeFilippes teaches of the second set of assays, "Figure 2 shows the results when the same 1247 base sequence is inactivated as a template for amplification of a 994 base sequence within that template . . . In this case also, *AluI* did not always completely inactivate the template." Page 28, column 3, lines 2-11. Thus, DeFilippes teaches that in each test assay performed to determine the usefulness of *AluI* to decontaminate PCR reagents in a PCR method, *AluI* failed. Because DeFilippes teaches that *AluI* is ineffective at completely digesting and therefore decontaminating reagents for PCR, DeFilippes teaches away from using *AluI* as a restriction endonuclease for digesting PCR reagents in a method of performing PCR.

One of ordinary skill in the art would not have been motivated to modify Steinman's method at the step of digesting PCR reagents with a restriction endonuclease to employ *AluI* as the restriction endonuclease as taught by DeFilippes. DeFilippes teaches away from using *AluI* to digest PCR reagents because DeFilippes teaches that *AluI* cannot be relied upon to cleave the DNA that contaminates PCR mixtures. See DeFilippes at page 28, column 2, line 16 to column 3, line 1 and at page 28, column 3, lines 2-11, quoted above. Because DeFilippes teaches away from the use of *AluI* as a restriction endonuclease for digesting PCR reagents, one of ordinary skill in the art would not have been motivated to use *AluI* in Steinman's PCR method. Thus, one of ordinary skill in the art would not have been motivated to combine Steinman and DeFilippes to arrive at the claimed invention and the *prima facie* case of obvious of claims 8-10, 15 and 18, dependent from claim 2, must fail.

Applicant respectfully requests withdrawal of this rejection.

The Rejection of Claims 5-7 and 11-22 Under 35 U.S.C. § 103(a)

Claims 5-7 and 11-22 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Steinman in view of Hoshina *et al.* (U.S. Patent No. 5,571,674; "Hoshina").

Claims 5-7 and 11-22 depend from claim 2. Claim 2 recites a method of performing PCR which comprises a step of "digesting reagents for polymerase chain reaction with AluI restriction endonuclease." Claim 5 recites the sample is a treated blood sample. Claim 6 recites that the blood sample is from a patient suspected of systemic bacteremia. Claim 7 recites that the primers comprise sequences as shown in SEQ ID NO:1 and SEQ ID NO:2. Claim 11 recites that the blood sample was treated to extract DNA therefrom. Claims 12 and 13 recite that the sample is urine and cerebrospinal fluid, respectively. Claim 14 recites that the primers hybridize to at least 10 eubacterial species' DNA in regions which are highly conserved. Claim 15 recites that the primers hybridize to 16S RNA genes. Claims 16-19 each recite a further step of the method of claim 2 for identifying a bacterial species as a source of the templates. Claim 20 recites that the Taq DNA polymerase is not active under the conditions used for the step of digesting. Claims 21 and 22 recite that the amplified product comprises at least 1 or 2 recognition sites for the AluI restriction endonuclease, respectively. Hoshina is cited as teaching the recitations of each of these dependent claims.

To establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). The combination of Steinman and Hoshina fails to teach or suggest all the elements, *i.e.*, "digesting reagents for polymerase chain reaction with AluI restriction endonuclease," as recited

in claim 2, the claim from which rejected claims 5-7 and 11-22 depend.

Steinman teaches a method of performing PCR. Steinman, however, does not teach or suggest digesting reagents for PCR with AluI restriction endonuclease. The Office Action acknowledges that Steinman fails to teach or suggest digesting reagents for PCR with AluI restriction endonuclease. See the Office Action at page 5, line 9, quoted above.

Hoshina, like Steinman, fails to teach or suggest digesting reagents for PCR with AluI restriction endonuclease. In fact, Hoshina does not teach restriction endonuclease digesting reagents for PCR. Hoshina teaches first mixing reagents for PCR with template DNA and second amplifying the target DNA. Hoshina teaches,

The invention further concerns a highly sensitive method for detecting the presence of bacteria or protozoa in a sample of a isolated DNA comprising contacting the sample with DNA oligomers which are polymerase chain reaction primers and are complementary to DNA sequences encoding universal bacterial and protozoan 16S ribosomal RNA sequences, such universal sequences being located at the 3' and 5' ends of species-specific DNA encoding species-specific bacterial or protozoan 16S ribosomal RNA, under conditions suitable for a polymerase chain reaction so as to amplify the bacterial or protozoan species-specific DNA lying between the binding sites of the DNA oligomers, and detecting the resulting species-specific bacterial or protozoan DNA sequences.

Column 7, lines 21-34.

Thus, the combination of Steinman and Hoshina fails to teach or suggest "digesting reagents for polymerase chain reaction with AluI restriction endonuclease" as recited in claim 2 and dependent claims 5-7 and 11-22. The combination of Steinman and Hoshina thus fails to teach or suggest all the elements of claims 5-7 and 11-22. The *prima facie* case of obviousness of these claims must fail.

Applicant respectfully requests withdrawal of this rejection.

Respectfully submitted,

Date: November 20, 2005

By:
for

Rachel A. Kagan Reg. No. 32,141
Michelle Holmes-Son
Registration No. 47,660

Customer No. 22907